THE VITALITY OF, AND CHANGES UNDERGONE BY, TRYPANOSOMES IN THE CADAVER OF THE ANIMAL HOST

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PLATE V

Yakimoff and Kohl (1907) found that *Trypanosoma evansi* preserved its vitality in the dead body of an animal for a maximum period of twenty-seven hours. Musgrave and Clegg, working with the same trypanosome, stated that motile parasites were not usually found two hours after death, but that in exceptional and rare cases, living parasites were found after sixteen hours. The same observers noted that blood was rarely infective after twenty-four hours, but that in one case the blood of an animal which had been dead forty-eight hours was still infective

In regard to human beings who had died of trypanosomiasis, Gray (1907) observed that actively motile trypanosomes could generally be found somewhere in the dead body within a few hours after death, but that they were seldom found in the blood.

The experiments detailed below were carried out in order to determine how long trypanosomes remained alive and infective in animals (rats and mice) which had been killed during an infection with trypanosomes, and also to study the changes which the parasites underwent in such dead animals.

Method of carrying out the experiments. For the first five experiments the following procedure was adopted. An infected rat was killed when the parasites first became numerous in its blood. It was kept at laboratory temperature, the experiments being carried out during the months of July, August and September. At the time of death films were made, both fresh and stained preparations. After a stated interval the animal was opened, and in addition to making fresh and stained preparations from the heart blood, an

inoculation was made into a rat or mouse. At definite periods, fresh and stained films were made and inoculations done. Thus, several examinations and inoculations were made from each animal. The appearances in fresh film and stained preparation were noted, and the conditions found at each period were compared with the results of the inoculations made. In the last four experiments a somewhat different plan was followed. A number of rats or mice, generally six, of equal size, was inoculated on the same day with equal doses of trypanosome-containing blood. These animals were killed simultaneously as soon as the last of them had passed the incubation period and presented a fair infection. At the time of death, therefore, some of these animals were fairly, others heavily, infected. The dead bodies were then left at laboratory temperature for certain periods, after which they were opened, and blood, taken from the heart, was utilized to make fresh films, stained preparations, and inoculations into animals. In these experiments each animal was used only once for inoculation purposes. It was possible thus to observe how long living trypanosomes remained microscopically evident in the blood of the dead animals, to note changes in character of the parasites in fresh and stained conditions, and also to fix a definite time after which the blood containing the parasites was no longer infective on inoculation. Intraperitoneal injection was used throughout the experiments, a definite amount of blood mixed with 1/4 c.c. of a citrated physiological salt solution being injected on each occasion.

Strains of trypanosome used. The majority of the experiments were made with *T. gambiense* and *T. rhodesiense*, a smaller number being made with *T. brucei*, *T. equinum* and *T. pecorum*.

The vitality of the trypanosome in the cadaver. In the fresh films, a hundred and fifty fields (Zeiss objective DD, ocular No 4) were examined before the blood was considered negative. From Table I it will be seen that in the blood of dead rats living trypanosomes were discovered up to twenty-one hours in the case of *T. equinum*, in the case of *T. brucei* up to twenty hours, up to twenty-four hours in the case of *T. pecorum*, up to twenty-nine hours in the case of *T. rhodesiense;* in the blood of dead mice living trypanosomes were found up to forty-eight hours in the case of *T. gambiense*.

A. CHANGES IN THE TRYPANOSOMES AS SEEN IN FRESH FILMS

(*a*) *Decrease in numbers.* The first fact observed was the decrease in the numbers of the active living parasites after the death of the animal host. Taking for example Experiment I, *T. equinum.* At death, the note is, fifty living to a field, after four hours ten to a field, after ten hours five to a field, after twenty-one hours one to a field, after twenty-eight hours none found. This fairly steady decrease was not always noted. In one experiment, No 11, a rat infected with *T. rhodesiense*, a most markedly rapid decrease was observed. At death there were about two hundred living parasites to a field, after four hours only two to a field, after twelve hours one to five fields, after twenty hours one to twenty fields, after thirty-six hours none found. In this case it will be seen that the decrease is extremely rapid during the first few hours after death. There is a point of considerable interest about this particular decrease which will be returned to later, in referring to the stained films.

(*b*) *Changes in appearance.* The changes consisted chiefly of swelling up of the protoplasm, the posterior end being mostly affected, resulting in the production of club-like, skate-shaped, and globular forms. A granular appearance was also marked in these altered parasites, beginning immediately after death.

(*c*) *Changes in motility.* As the trypanosomes became bulbous and distorted, their movements became slower and finally ceased. Frequently, as they slowed down large clumps of agglutinated trypanosomes were found, many of the individuals still moving fairly actively. Many parasites, however, often of granular appearance, became immobilized without loss of shape, and could be seen floating amongst the corpuscles. In certain cases, although the number of active trypanosomes had decreased very greatly, those which persisted and retained their normal shape, moved with marked activity, even apparently in excess of the normal. This was particularly noted in *T. gambiense* in mice, after forty-eight hours.

Infectivity of the blood of dead animals. Table I shows the maximum period of infectivity of the blood from the cadaver of the trypanosomiasis animals. In the blood of dead rats *T. rhodesiense* was infective after forty-two hours, *T. gambiense* after twenty-nine hours. In the blood of dead mice both *T. rhodesiense* and

T. gambiense were infective after forty-eight hours. In Table II, where the experiments are analysed in greater detail, are given the period at which inoculation was done after the death of the animal host, whether live trypanosomes were seen or not, the incubation period, and time of death in those inoculated animals which became infected.

It will be observed that several inoculated animals died so soon from various causes, that the parasites had not time to develop. In this connection it may be noted that in no case where living trypanosomes were seen at the time of inoculation, did infection fail to occur, in an animal which lived for a reasonable time.

Number of animals infected after varying periods. In Experiment VI, *T. rhodesiense*, each of three rats inoculated with blood from three dead rats which had lain twenty-nine hours at laboratory temperature, became infected, and one out of three when the dead rats had lain forty-two hours. In Experiment VII, *T. gambiense*, two of three rats inoculated with blood of rats twenty-nine hours dead became infected, none of three rats became infected where blood was used from bodies which had lain forty-two hours. In Experiment VIII, *T. rhodesiense*, where the blood was taken from mice forty-eight hours dead, one mouse out of two which lived twenty days became infected. In Experiment IX, *T. gambiense*, the blood of mice dead forty-eight hours was used for inoculation. The only mouse which lived twenty days became infected. Had the others lived it is probable that several more would have been infected, as three out of the five original mice had living parasites in their blood after they had been dead forty-eight hours.

B. CHANGES IN THE TRYPANOSOMES AS SEEN IN STAINED PREPARATIONS

Soon after death a granular change was seen in many of the parasites. The granules appeared quickly, many forms being filled with granules even within one hour after death. The conditions present after the death of the animal host may be divided into two classes, both of which were observed in varying degrees.

(1) *Changes accompanied by loss of form.* Some trypanosomes became granular, the nucleus being broken up into small staining

globules, and the whole protoplasm being filled with dark stained granules, contrasting with the paler protoplasm of the parasite. Other forms were seen in which apparently these granules were greatly reduced in number, only one or two dots being seen in the region where the nucleus was.

Non-nucleated forms. Forms were seen in which, although the protoplasm, blepharoplast and flagellum, and outline of the parasite appeared perfect, there was no trace of nucleus or granules to be seen. These non-nucleated parasites were first observed in a rat infected with *T. pecorum*, which was dying swarming with parasites. Later a search for them proved their presence in the living rat infected with the strains of *T. gambiense*, *T. rhodesiense*, *T. equinum*, *T. brucei*, *T. dimorphon*, at similar times. They were then looked for and found in these experiments, but although found with ease soon after death, it was not easy to discover them later on. They did not appear to be parasites from which, as a result of fracture or pressure, the nucleus had been displaced, because in such cases one could generally discover traces of the fractured and displaced nucleus, and the parasites did not present a clear outline. Nor were they the result of imperfect staining, because trypanosomes immediately adjacent to them presented perfectly stained nuclei. It seems probable that they were the result of a process of granular disintegration of the nucleus, after which solution or extrusion of the granules had followed. This mode of degeneration, however, if it is degeneration, was by no means a common one in these experiments.

(2) *Changes accompanied by loss of form.* The majority of the trypanosomes soon after death became swollen and distorted. The component parts, still clearly visible and well stained, lost their relative positions, so that in many the nucleus and blepharoplast came to lie very close together, rounded bodies being formed, with the flagellum encircling them.

Definite degeneration forms. The term 'degeneration is used here to signify only those changes which were present in trypanosomes in the blood of a dead animal, at a time when the blood containing such altered trypanosomes failed, on inoculation, to infect rats and mice. The test, therefore, made use of in the experiments, to decide the question whether changes observed in

the trypanosomes indicated degeneration or not, was the effect of inoculation into these animals.

The various parts of the parasite disappeared irregularly, giving rise to an infinite variety of forms, among which the round form predominated, a protoplasmic circular mass containing the nucleus and blepharoplast or possibly granules. In many no flagellum could be made out, in which case the smaller body often seen with the nucleus was probably granular in origin. A further step consisted in the isolation of those nuclei which persisted and the detachment of the flagellum with the blepharoplast. Masses of nuclei were found which represent the remains of agglutinated groups of trypanosomes. The variety of forms presented by the various degenerating portions of trypanosomes is enormous. A few of them are shown in Plate V. All the forms represented in this Plate, Part B, are taken from blood after it had ceased to be infective on inoculation, and are, therefore, 'degeneration' forms in the sense of the definition given above. That is to say, blood which contained, in many instances in enormous numbers, these rounded forms, somewhat resembling the appearance of described 'resistant' forms, failed to infect rats and mice.

In practically all the experiments, careful search of films taken after the blood had ceased to be infective, revealed the presence of a few fairly well-stained, well-shaped parasites.

The decrease of the parasites in Experiment II, T. rhodesiense. Above, in dealing with the conditions seen in fresh films in this case, it was noted that a very rapid decrease of the trypanosomes occurred within a few hours of death. Examination of the stained films showed that this decrease was remarkable in one respect. The stained film, taken at the time of death, when there were about two hundred parasites to the field in the fresh film, showed a very large preponderance of the usual long, free flagellated trypanosomes, and a small proportion of short, stumpy forms, some of these having the posterior nucleus which is characteristic of this strain. The proportion of long, free flagellated forms to short, stumpy forms, was at this time eighty to one. Within four hours, however, this proportion had been reduced to five to one. After twelve hours the proportion of long to short had sunk to one long to twenty short, and after twenty-two hours, of fifty trypanosomes seen, only two

were long forms. Further, it was observed that the short, stumpy forms were more perfect in their appearance and staining reaction than the long forms. They appeared, therefore, in this experiment, to have a markedly greater power of resistance to the process of disintegration than did the long forms. As regards the other experiments this peculiarity was not noticeable.

The incubation period in animals inoculated with blood from the cadaver. Yakimoff and Kohl (1907) found that the incubation period was increased when cadaver blood was used, but that the sooner after death the inoculation was made the less marked was the lengthening of the incubation time. Somewhat similar results were obtained in these experiments. A reference to Table II will show that the maximum incubation period for *T. gambiense* was twenty days in a mouse (blood forty-eight hours old), and for *T. rhodesiense* nineteen days in a rat (blood twenty-nine hours old). It will be observed, however, that one mouse, Experiment VIII B, inoculated with *T. rhodesiense* blood, forty-eight hours old, presented an incubation period of eleven days only.

In several individuals, after a prolonged period, the trypanosomes became numerous in the blood very rapidly, more so than in the usual infections with ordinary infected blood.

CONCLUSIONS

(1) *T. gambiense* and *T. rhodesiense* can remain infective in the blood of the dead animal host for forty-eight hours.

(2) This infectivity need not be attributed to any specially formed 'resistant bodies,' as living trypanosomes were found by the microscope (e.g., *T. gambiense*) in three out of five animals which had been dead forty-eight hours.

(3) In case of accident, in which all the animals holding a strain of trypanosomes died at once, it would be worth while inoculating up to forty-eight hours, probably even longer, after death. For such inoculations a large series of animals should be used, as several of them would almost certainly die before the incubation period was over.

REFERENCES

Yakimoff and Kohl (1907). 'De la vitalité de Trypanosomes dans les cadavres.' Archives des Sciences Biologiques (St. Petersbourg), Nos. 4, 5, pp. 351-358.

Musgrave and Clegg. 'Trypanosoma and Trypanosomiasis,' p. 46, *vide* Bull. Sleeping Sickness Bureau, 1909, No. 8, p. 294.

Gray and Tulloch (1907). Reports of the Sleeping Sickness Commission of the Royal Society, VIII, p. 17.

TABLE I.—To show, for the experiments done, the maximum duration of infectivity in blood of dead trypanosomiasis animals, kept at laboratory temperature.

No. of Experiment	Strain	Animal	Maximum time (in hours) post mortem after which—		Inoculations		
			(1) Living trypanosomes found in fresh film	(2) Blood infective	Animal	Incubation in days	Duration of disease in days
I	*T. equinum*	Rat	21	21	Rat	14	21
II	*T. rhodesiense*	Rat	22	22	Rat	8	11
III	*T. brucei*	Rat	20	29	Rats (1)	11	38
					(2)	7	29
IV	*T. gambiense*	Rat	11	11	Mice (1)	5	10
					(2)	5	10
V	*T. pecorum*	Rat	24	24	Rats (1)	8	12
					(2)	11	13
VI	*T. rhodesiense*	Rat	29	42	Rat	11	32
VII	*T. gambiense*	Rat	None seen	29	Rats (1)	7	20
					(2)	7	9
VIII	*T. rhodesiense*	Mouse	None seen	48	Mouse	11	20
IX	*T. gambiense*	Mouse	48	48	Mouse	20	31

Table II.—An analysis of the foregoing experiments showing the period after the death of the animal at which inoculations were done, and the results of the inoculations.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
I ...	*T. equinum*—						
	Rat	10	+	Rat	10	20th	
		21	+	Rat	14	21st	
		30	—	Rats (1)	—	42nd	Trypanosomes never found in blood.
				(2)	—	11th	,, ,, ,,
		36	—	Rats (1)	—	33rd	,, ,, ,,
				(2)	—	21st	,, ,, ,,
II ...	*T. rhodesiense*—						
	Rat	12	+	Rat	5	11th	
		22	+	Rat	8	11th	
		36	—	Rats (1)	—	1st	Rat died without apparent cause.
				(2)	—	1st	,, ,, ,,

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
— Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
III ...	*T. brucei*—						
	Rat	29	—	Rats (1)	11	38th	
				(2)	7	29th	
IV ...	*T. gambiense*—						
	Rat	11	+	Mice (1)	5	10th	
				(2)	5	10th	
		24	—	Mice (1)	—	7th	Trypanosomes not found.
				(2)	—	6th	,, ,,
		36	—	Mice (1)	—	4th	,, ,,
				(2)	—	4th	,, ,,
V ...	*T. pecorum*—						
	Rat	24	+	Rats (1)	8	12th	
				(2)	11	13th	

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
— Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
VI ...	*T. rhodesiense*—						
	Rats :—						
	A	29	+	Rat	19	24th	
	B	29	+	Rat	12	25th	
	C	29	—	Rat	10	23rd	
	D	42	—	Rat	—	—	Alive 35th day. Tryps. never seen.
	E	42	—	Rat	—	30th	
	F	42	—	Rat	11	32nd	Tryps. became numerous on 18th day, then disappeared and returned on 23rd, and became numerous again at death.
VII ...	*T. gambiense*—						
	Rats :—						
	A	29	—	Rat	7	20th	
	B	29	—	Rat	7	9th	
	C	29	—	Rat	—	9th	Tryps. never seen.
	D	42	—	Rat	—	9th	
	E	42	—	Rat	—	—	Alive 38th day. Tryps. never seen.
	F	42	—	Rat	—	18th	Tryps. never seen.

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
— Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	•	Animal inoculated	Incubation in days	Day of death	Remarks
VIII ...	*T. rhodesiense*—						
	Mice :—						
	A	48	—	Mouse	—	—	Alive 40th day. Tryps. never seen.
	B	48	—	Mouse	11	20th	,, ,,
	C	48	—	Mouse	—	12th	Tryps. never seen.
	D	48	—	Mouse	—	7th	,, ,,
	E	48	—	Mouse	—	26th	,, ,,
	F	48	—	Mouse	—	—	Alive 40th day. Tryps. never seen.
IX ...	*T. gambiense*—						
	Mice :—						
	A	48	—	Mouse	—	2nd	Septicaemic infection.
	B	48	+	Mouse	—	2nd	,, ,,
	C	48	+	Mouse	—	9th	Tryps. never seen.
	D	48	—	Mouse	—	3rd	,, ,,
	E	48	+	Mouse	20	31st	

• + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
— Signifies living trypanosomes not found.

EXPLANATION OF PLATE V

Magnification 2,000 to 2,100 diameters.

PART A

Nos. 1, 2, 7, 8. *T. rhodesiense* in rat soon after death.

No. 3. *T. gambiense* in mouse 48 hours after death.

No. 4. *T. rhodesiense* in rat 29 hours after death.

No. 5. *T. rhodesiense* in rat 48 hours after death.

No. 6. *T. rhodesiense* in rat 5 hours after death.

PART B

Nos. 1, 7, 17. *T. rhodesiense* in mouse 48 hours after death.

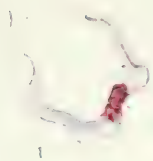
Nos. 2, 5, 6, 16. *T. rhodesiense* in rat 48 hours after death.

No. 3, 8, 9, 11, 12. *T. rhodesiense* in rat 42 hours after death.

Nos. 4, 10, 13, 14, 15. *T. gambiense* in rat 42 hours after death.

PLATE V

PART A.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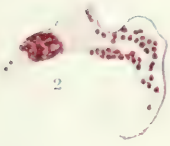
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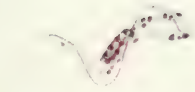
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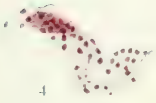
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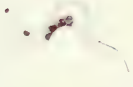
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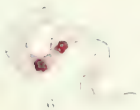
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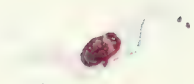
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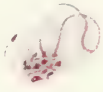
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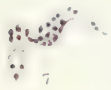
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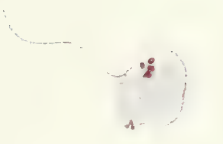
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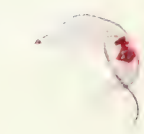
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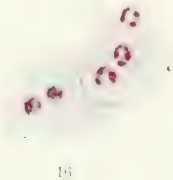
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